

BASIC RESEARCH STUDIES

Differential proteolytic activity and induction of apoptosis in fibrous versus atheromatous plaques in carotid atherosclerotic disease

Theresa Jacob, PhD,^a Enrico Ascher, MD,^a Anil Hingorani, MD,^a Yevgeniy Khandros,^a Boris Tsémekhin, MD,^a Linda Zeien, MD,^b and Yilmaz Gunduz, MD,^a Brooklyn, NY

Purpose: Atherosclerotic plaque instability may be a contributing factor to plaque complications, such as rupture, thrombosis, and embolization. Of the two types of plaques, atheromatous and fibrous, the atheromatous type has been reported to be vulnerable and unstable. This instability may be related to changes in the cell cycle and extracellular matrix degradation. Apoptosis may weaken the plaque structurally. In addition, alteration of the cellular component may lead to imbalances in associated proteolytic activity. Our study was designed to compare the two types of plaques in terms of apoptosis, apoptosis-inducing factors, namely Fas/CD95/APO-1 and CPP-32/YAMA/caspase-3, and proteolytic activity.

Methods: Carotid artery plaques were obtained from patients undergoing endarterectomy and were classified as either atheromatous or fibrous on the basis of established criteria. Histologic study included hematoxylin and eosin staining, Verhoeff's van Gieson elastin staining, and trichrome staining. Detection of apoptosis was performed with the TUNEL assay. Immunohistochemical studies were performed to localize the expression of CPP-32/YAMA and Fas/CD95. Gelatin zymography was used to compare proteolytic activity levels in the two types of plaque.

Results: Apoptosis was significantly higher ($P < .001$) in atheromatous plaques ($4.90\% \pm 1.27\%$ [SEM]) as compared with fibrous plaques ($0.86\% \pm 0.46\%$ [SEM]). Zymography demonstrated elevated levels of proteinases in atheromatous plaques. Immunohistochemistry revealed significant increases in the expression of Fas/CD95 ($P < .04$) and CPP-32/YAMA ($P < .001$) in atheromatous plaques as compared with that in fibrous plaques.

Conclusions: This is the first study comparing molecular factors that render atheromatous plaques more susceptible to rupture than fibrous plaques. The higher number of apoptotic cells seen in atheromatous plaques as compared with fibrous plaques could contribute to their greater instability. Immunoreactivity to cytoplasmic death domain, Fas/CD95 and CPP-32/YAMA, a prominent mediator of apoptosis, was consistent with the numbers of apoptotic cells detected. The increased levels of proteolytic activity in atheromatous plaques may make these plaques more prone to rupture. These data identifying some of the molecular events and biochemical pathways associated with plaque vulnerability may help in the development of new strategies to prevent plaque rupture. (J Vasc Surg 2001;33:614-20.)

In carotid atherosclerotic disease, fibrous and atheromatous plaques have exhibited different levels of stability.¹⁻³ Fibrous plaque is made up of more than 70% collagen-rich tissue that is thought to stabilize and prevent rupture,⁴ whereas atheromatous plaques, characterized by having high lipid content, thin fibrous cap, and abundant macrophages, are known for their instability.⁵ Although data support the clinical differences between these two

types of plaques, few studies have investigated the different types of plaques by use of modern biochemical and molecular biological techniques.

Previous studies by us, as well as by others,^{6,7} have demonstrated increased levels of apoptosis and signaling molecules of the apoptotic cascade in atherosclerotic plaques as compared with normal arterial tissue. Specifically, changes in the expression of the members of the Bcl-2 family, p53, MDM2, CPP-32, and cyclin D1 have been reported.⁶⁻⁹ It has been proposed that apoptosis, especially of the smooth muscle cells (SMCs) in the fibrous cap and the underlying media, weakens the plaque structurally to the point of rupture whereas death of macrophages and that of other cells contribute to the formation of soft plaque cores and therefore make them vulnerable.¹⁰⁻¹² However, the magnitude of apoptosis and the signaling molecules involved in apoptosis in fibrous versus atheromatous plaques have not been examined.

Plaque rupture may also be affected by the stability of extracellular matrix (ECM). Remodeling of the arterial

From the Division of Vascular Surgery^a and the Department of Pathology,^b Maimonides Medical Center.

Competition of interest: nil.

Supported by a grant from Maimonides Research and Development Foundation.

Presented at the Annual Meeting of Society for Clinical Vascular Society, Rancho Mirage, Calif, March 18, 2000.

Reprint requests: Enrico Ascher, MD, Division of Vascular Surgery, Maimonides Medical Center, 4802 10th Ave, Brooklyn, NY 11219.

Copyright © 2001 by The Society for Vascular Surgery and The American Association for Vascular Surgery

0741-5214/2001/\$35.00 + 0 24/6/111802
doi:10.1067/mva.2001.111802